EFFECTIVENESS OF AN UNDEFINED MUCOSAL COMPETITIVE EXCLUSION TREATMENT TO CONTROL *SALMONELLA* IN TURKEYS DURING BROODING

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SUMMARY

Mucosal competitive exclusion (MCE) cultures generated from the mucosal scrapings of healthy adult turkeys were administered to commercial turkey poults. MCE-treated poults were placed on tom and hen farms with paired untreated control poults in adjacent houses. After 6 wk in the brood house, cecal droppings from control and treated flocks were collected and analyzed for the presence of salmonellae. Salmonellae were detected in 14 of 30 cecal droppings (47%) from control flocks and from only 1 of 30 (3.3%) droppings from the treated poults. This study demonstrated that mucosal competitive exclusion could be used to effectively control salmonellae in a commercial field trial of young turkey flocks.

Key words: Brood, competitive exclusion, mucosal competitive exclusion, *Salmonella*, turkeys
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DESCRIPTION OF PROBLEM

In 1973, a possible means for reducing the incidence of salmonellae in poultry flocks by using undefined mixtures of gut microorganisms was first described [1, 2]. The newly hatched chick has an undeveloped gut microflora and as such is susceptible to intestinal colonization by salmonellae. By introducing intestinal bacteria from adult birds, the young chick can be protected against salmonellae colonization. This process has come to be known as competitive exclusion. Most of the previous studies with competitive exclusion have been primarily concerned with the control of salmonellae in chickens [3, 4, 5, 6, 7, 8]. However, some past studies with competitive exclusion have also been performed with turkeys [9, 10, 11, 12, 13, 14]. The objective of this study was to determine the effectiveness of an undefined competitive exclusion culture made from the microorganisms present in the mucosa of adult turkeys to prevent salmonellae contamination of male and female young poults in a commercial setting.

MATERIALS AND METHODS

Mucosal competitive exclusion (MCE) cultures were produced with cecal epithelial wall scrapings of healthy adult turkeys that were inoc-

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ulated into tubes of pre-reduced brain heart infusion (BHI) broth [15], which was then incubated for 48 h at 37°C [16]. Initial cultures were subcultured anaerobically three times by transferring 1 mL of incubated culture with a syringe into a fresh tube of Hungate-sealed BHI broth. Efficacy of MCE was determined by comparing MCE-treated and control (untreated) poults. Salmonellae-free day-of-hatch poults (determined by failure to recover the organism from enrichment of the straw mats used in transport of day-old poults) were treated by gavage with 0.2 mL of a 48-h MCE subculture. These poults were divided into groups of 10 poults each and placed in isolation units (IU). Two days later, the treated and control poults were challenged by gavage with 10^4 or 10^6 cfu of a nalidixic acid resistant strain of *Salmonella typhimurium* per poult with one challenge level only per IU. The two challenge levels were chosen to demonstrate how effective the cultures were at medium and high levels of *Salmonella* challenge.

One week later, poults were killed by cervical dislocation, and their ceca were aseptically removed to quantify the number of *S. typhimurium* per gram of ceca and cecal contents. These counts were used to generate a colonization factor (CF) as described by Bailey et al. [17]. The mean $\log_{10}$ colony-forming units per gram of cecal material was determined for all samples within a treatment group for each challenge organism. This calculation was described by Pivnick et al. [4] for infection factor (IF), but because the colonization studied in this study was commensal and not pathogenic the term colonization was favored over infection. Rate of colonization was calculated by dividing the number of birds colonized by the total number of birds challenged with the organism. Protection factor (PF) as described by Pivnick et al. [4] is the ratio of the CF for an untreated group of chicks to the CF for a treated group within the same experiment. A larger PF provides more effective treatment against colonization by the challenge organism. This method is widely accepted for calculating competitive exclusion culture efficacy.

The MCE culture that was most effective in the IU trials was used to generate the larger volumes necessary for the commercial field trial. These data were not included, but the culture chosen was 100% effective in preventing *Salmonella* colonization in the poults challenged at both levels in the IU trials. Large volumes of MCE for this commercial field trial were produced by inoculating 1-L bottles of prewarmed, pre-reduced anaerobic BHI broth with 1 mL of a 48-h subculture of MCE. Bottles were equipped with Hungate caps connected to gas-release water traps and then incubated in a hatchery incubator cabinet at approximately 37°C for 36 to 48 h before use.

The MCE cultures were applied in two stages. The first treatment consisted of spraying undiluted MCE culture on newly hatched poults using a portable pressure garden sprayer when the poults were 50 to 75% hatched, which resulted in each poult and unhatched egg receiving approximately 0.2 to 0.3 mL of MCE. Treated and control eggs were maintained in separate hatching cabinets. The secondary phase of treatment applied in the broiler houses provided a 1:10 dilution of MCE in 3.8-L plastic drinker jars as the poult's first drinking water. Drinkers were left in place until all of the MCE culture had been consumed (approximately 4 h). The drinker to poult ratio was 1:200. On average, each poult consumed 10 mL of the diluted MCE.

After 6 wk of brooding, 15 fresh cecal droppings were obtained from the control and from each treated house. After receipt in the laboratory, samples were mixed with buffered peptone [18] and incubated for 24 h at 37°C. Next, the procedure involved enrichment in TT broth [19], isolation on brilliant green sulfa agar and modified lysine iron agar [20], presumptive classification on lysine iron agar slants, and serological confirmation with Poly-O and Poly-H antisera [21]. All confirmed isolates were serotyped by the USDA Veterinary Diagnostics Laboratory [22].

Results and Discussion

The MCE culture was shown to be very effective in controlling salmonellae colonization of young commercial turkeys during the brood period (Table 1). At 6 wk of age, 14 out of 30 (47%) of untreated poults were shedding salmonellae in the fecal material compared with 1 of 30 (3%) MCE-treated poults. The treatment was effective for male and female poults. For the

TABLE 1. Efficacy of an undefined mucosal competitive exclusion treatment to control salmonellae in turkeys during brooding

		TREATMENT	
AGE (wk)	SEX	Control	MCE[A]
6	Male	6/15 (40.0%)	0/15 (0.0%)
6	Female	8/15 (53.3%)	1/15 (6.7%)
Total		14/30 (46.7%)	1/30 (3.3%)

[A]MCE = mucosal competitive exclusion.

males 6/15 (40%) of the untreated poults were infected with salmonellae, and none of the MSC-treated poults were infected [0/15 (0%)]. For the females, 8/15 (53.3%) of the untreated poults were infected with salmonellae and only 1/15 (6.7%) of the MSC-treated poults were infected. Previous European studies that tested undefined competitive exclusion cultures made from chicken were not very effective in protecting poults from salmonellae [23, 24]. One of the studies [24] reported greater than 40% salmonellae infection of the treated poults. The current study suggested that when an undefined culture was made from turkeys to subsequently treat turkeys, the efficacy was far greater than when a chicken culture was used to treat turkeys.

Competitive exclusion treatment with turkeys has been shown to be ineffective against *Salmonella* that are introduced in the hatchery [25, 26, 27, 28]. However, in this study, the three *Salmonella* serovars (*S. arizona*, *S. heidelberg*, and *S. kentucky*) isolated from hatchery samples (data not shown) were not found in any of the 6-wk samples from treated males or females. One explanation may be that comparatively low levels of hatchery contamination may be controlled by MCE. The same serovars were isolated from control and treated females (*S. agona*, *S. albany*, *S. muenchen*, *S. muenster*, and *S. typhimurium*). For the males, *S. hadar*, *S. senftenberg*, and *S. typhimurium* were isolated from the controls and *S. reading*, *S. senftenberg*, and *S. typhimurium* were isolated from the one positive sample in the treated group. In this study, the MCE culture was effective in a commercial setting even though there were *Salmonella* present in the hatchery.

Conclusions and Applications

1. This study demonstrated that an undefined mucosal competitive exclusion culture could effectively control salmonellae in male and female poults in a commercial setting.
2. Research directed at reducing the intestinal carriage of salmonellae in turkeys should reduce human exposure associated with the consumption of turkey and turkey products.
3. Competitive exclusion cultures made from turkeys were shown to be more effective in reducing salmonella than had previously been reported for competitive exclusion cultures made from chickens.

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